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I, KIM MARSHALL, MANAGER EXAMINATION SUPPORT AND SALES, hereby certify that the annexed is a true copy of the Provisional specification in connection with Application No. PP 2428 for a patent by ANUTECH PTY LTD filed on 18 March 1998.

I further certify that the above application is now proceeding in the names of THE AUSTRALIAN NATIONAL UNIVERSITY and AUSTRALIAN WATER TECHNOLOGIES pursuant to the provisions of Section 104 of the Patents Act 1990.

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AUSTRALIAN NATIONAL UNIVERSITY
and AUSTRALIAN WATER TECHNOLOGIES

A U S T R A L I A

Patents Act 1990

PROVISIONAL SPECIFICATION

for the invention entitled:

"Method of water purification"

The invention is described in the following statement:

METHOD OF WATER PURIFICATION

The present invention relates to a method for the purification of water. More particularly, the present invention relates to the removal of biological contaminants from water.

The presence of biological pathogens in water bodies, such as rivers, dams, seawater and swimming pools, where human contact is likely to occur, or, in water intended for human or animal consumption, is a hazard which may potentially result in illness, disability or even death if these pathogens are inadvertently ingested by humans or animals. Accordingly, there exists a variety of methods for their removal so as to render contaminated water safe for human consumption. Known methods of removing pathogens from contaminated water include mechanical filtration, *i.e.* physical exclusion based on the size of the biological pollutants, chemical treatment such as chlorination and ozonation and electrolysis which generates oxidants fatal to the pathogens.

One such pathogen is *Cryptosporidium* which can last up to six months in a moist environment and commonly infects public swimming pools. The spread of infection is rapid and the presence of the pathogen is often only detected once a number of people are infected. Whilst in most cases this may only result in gastric upsets infection can pose a more severe danger, in the form of dehydration, for small children or the elderly. In addition, those who are already immunocompromised, such as people who are HIV positive are also at serious risk.

The present inventors have carried out microelectrophoretic studies on *Cryptosporidium* oocysts (Figure 1). These studies indicated a negative surface potential of around -27mV in distilled water at pH 5.7. In addition, measurement of the zeta potential over a range of pH values (Figure 2) gave a pKa value of 2.5 indicating the presence of negatively charged groups on the oocyst surface such as carboxylate or phosphate groups. The subject invention

now provides a new method for removing pathogens from water which relies on the negative surface potential or the presence of negatively charged functional groups on the surface of a biological species.

Accordingly, the present invention provides a method for the removal of biological species possessing a negative surface charge or negatively charged functional groups on the surface from water comprising the step of contacting the water with a medium for a time and under conditions such that the biological species are adsorbed onto said medium.

Preferred biological species for removal by the method of the invention are human pathogens which include *Cryptosporidium* and *Giardia*. The negative surface charge on the biological species may be partial, localised or a net overall charge. The incidence of negatively charged functional groups on the surface of the biological species may be partial, localised or a net overall distribution.

Thus suitable media for use in this invention are those which possess an opposite surface potential to the negatively charged surface of the pathogen, that is, media which possess a positive surface charge in water. The positive surface charge on the medium may be partial, localised or a net overall charge.

As used herein, the terms "adsorb" and "adsorption" may refer to either electrostatic adsorption or chemisorption.

Without being limited by theory, it is believed that the electrical charge possessed by colloidal inorganic oxides in aqueous suspension can be attributed by two mechanisms:

(a) amphoteric dissociation of surface MOH groups (i.e. "the absorption of protons or hydroxyls on to an amphoteric site":

$$MOH_2^+ \xleftarrow{H^+} MOH \xrightarrow{OH^-} MO^- + H_2O$$

(b) adsorption of hydroxylated metal species derived from the hydrolysis products of material dissolved from the solid:

$$M^n + xOH^- \rightarrow M(OH)_x^{\left((n-x)^+\right)}$$

$$M(OH)_x^{(n-x)^+} + OH^- \rightarrow M(OH)_{x+1}^{(n-x-1)^+}$$

$$M(OH)_x^{(n-x)^+} + H^+ \rightarrow M(OH)_{x-1}^{(n-x-1)^+} + H_2O$$

Both mechanisms give a strong pH dependence of the surface charge and the existence of an isoelectric point (iep). However, the quoted values for the isoelectric points of alumina is extremely variable ranging from 6.7 to 9.2. This observed variation could be a result of a number of factors such as: (1) the degree of hydration of the surface, (2) the crystallographic form of the alumina, (3) the history of the sample, i.e. whether the sample had been aged in solution or polished, and (4) the effect of specific adsorption of dissociable species.

One possible explanation for the different isoelectric points observed for the different crystallographic forms of alumina is that the relative surface densities of aluminium and oxygen groups will vary depending on the structure of the surface. Synthetic alumina has been reported as having an isoelectric point of 6.7 [1].

The interactions existing between charged surfaces in electrolyte solutions are of central importance in many physical and biological processes (i.e. cellular fusion, clay swelling and drinking water clarification to name but a few) influencing the structure, stability and rheological properties.

When surfaces are immersed in a high dielectric constant solvent such as water, the surfaces acquire a charge, either by the ionisation of the surface functional groups (e.g. carboxylate and silanol groups) or by the preferential adsorption of dissolution of a particular ion. Ions of like charge (co-ions) are repelled away from the surface whereas ions of opposite charge (counter-ions) are electrostatically attracted to the surface, their thermal motion prevents them from becoming bound and therefore result in a diffuse cloud of charge next to the surface. The charged surface layer and its diffuse cloud of counter-ions form what is known as the *"diffuse electrical double layer"*.

When two similarly charged surfaces approach one another there is a repulsive electrostatic force generated by the electrical double layer overlap, and a short range ubiquitous van der Waals (VDW) attractive force. The net interaction energy obtained from the sum of these two opposing forces forms the basis of a quantitative (DLVO) theory of colloidal stability.

However, close contact between oppositely charged surfaces can also result in the formation of chemical bonds between surface sites on the approaching surfaces. This is called chemisorption and typically occurs between carboxylate, phosphate and wide range of metal cations such as aluminium, calcium, iron *etc.* in natural systems.

For oppositely charged surfaces there is a strong electrostatic attraction combined with a short range van der Waals force. These forces pull oppositely charged colloids together into strong adhesive contact.

Accordingly, two preferred substrates for use as adsorption media in the invention are: alumina (Al_2O_3) and fluorspar ($Ca F_2$). Both materials have an opposite surface potential (i.e. positive) to that of the oocysts in solutions at pH below about 8, therefore presenting good substrates for the direct adsorption from aqueous solution under normal pH conditions.

Alumina is readily available in powdered form with different size ranges. This material can be packed into a suitable, high flow rate filtration cartridge and can, for example, be used as the final stage in a swimming pool pumping-filtration unit. It is unlikely that the levels of chlorine present will have any effect on the powdered alumina. The presence of low levels of aluminium in drinking water is considered a matter of concern but this should not apply to swimming pool water. In any case only very low levels of aluminium will be released at the pH range (about 7.2) used for pools.

Other aluminium based media suitable for use in the present invention are soluble aluminium salts or partially hydrolysed forms thereof. When such aluminium salts are used, as adsorption media, the pH is adjusted to a level such that an insoluble alumina hydrated network, possessing surface Al-OH groups forms, entrapping and adsorbing the negatively charged pathogens. Preferred pH levels for forming these extended alumina networks are in the range of about 6.2 to about 7.0.

Thus, it is envisaged that Al-OH containing species may be utilised in a number of ways, i.e. as alumina or as soluble aluminium salts.

In one embodiment, the method comprises: (1) the addition of aluminium salts to the water containing the negatively charged pathogens; (2) adjusting the pH such that an extended hydrated alumina network is formed (wherein steps 1 and 2 are optionally performed in reverse order); and (3) removing the resulting precipitated/coagulated particles ("floc") by any suitable means such as sedimentation or filtration.

Another embodiment contemplates the use of a filtration cartridge packed with alumina powder. Due to its low solubility, the use of alumina powder renders the aluminium unavailable, excluding it from normal biochemical and metabolic processes thereby reducing any health risks from purported links between the ingestion of aluminium and neurodegenerative diseases. Accordingly, alumina is a preferred "aluminium" medium over soluble aluminium salts when purifying potable water or in swimming pool management applications.

Fluorspar is another good substrate for *Cryptosporidium* removal, due to its combination of positive charge in water and the presence of calcium ions within the crystal structure which can ligand bond to carboxylate and phosphate groups at the oocyst surface. It can be used either in a direct precipitation/coagulation process, by producing colloidal calcium fluoride produced by mixing suitable soluble calcium and fluoride salts, or in powdered form, in packed high flow rate filtration cartridges. The powdered insoluble material is more acceptable for use in drinking water applications.

Iron based compounds, such as iron oxides, may also be suitable for use in this invention.

As the following Examples describe, *Cryptosporidium* oocysts are found to strongly adsorb onto alumina and fluorspar surfaces. This, therefore, allows the provision of a system to remove oocysts from water which could be designed using, preferably, powdered alumina or fluorspar in a cartridge filter.

The results indicate that once adsorption onto the alumina or fluorspar takes place, the oocysts are strongly resistant to desorption, even in solutions at high pH, where the surfaces will become negatively charged. Such an independence of pH is a process safeguard because the oocysts will not be released on the water due to small pH variations.

It appears fluorspar is a good substrate for *Cryptosporidium* oocyst adsorption. The adsorbed oocyst layer appears to be stable and resistant to desorption with pH, EDTA and NaF treatment. Without limiting the invention to theory, *Cryptosporidium* adsorption appears to be due to both an electrostatic attraction and chemisorption between the surface calcium ions and either the carboxylate or phosphate groups present on the oocyst surface.

The invention will now be described with reference to the following non-limiting Examples and Figures.

FIGURES

Figure 1 depicts an electron micrograph of a *Cryptosporidium* oocyst.

Figure 2 is a graphical representation of the change in zeta potential of *Cryptosporidium* oocysts in 10^{-3}M NaCl as a function of pH.

Figure 3 is a photograph of *Cryptosporidium* oocysts adsorbed onto polished alumina wafers.

Figure 4 schematically depicts a size comparison between *Cryptosporidium* oocysts and individual alumina or silica spheres.

Figure 5 schematically depicts the retention of *Cryptosporidium* oocysts onto packed and unpacked columns of silica and alumina.

Figure 6 is a photograph of the optically smooth surface of an alumina wafer having a few extraneous dust particles.

Figure 7 graphically depicts the permeate levels of *Cryptosporidium* oocysts adsorbed after

repeated washings through a silica column..

Figure 8 graphically depicts the permeate levels of *Cryptosporidium* oocysts adsorbed after repeated washings through an aluminium column.

EXAMPLES

Materials and Methods

The γ irradiated bovine *Cryptosporidium* oocyst samples were supplied by AWT at a concentration of 5×10^6 and 3×10^8 oocysts/ml in distilled water which was stored at 4°C prior to use.

The water used in this study was produced from tap water, which was fed through a Memtec Krystal Kleen™ unit using a three-stage purification process (prefilter, reverse osmosis and activated charcoal) before being distilled, collected and stored in a positive pressure, dust free laminar flow cabinet to prevent air borne contamination.

Pure alumina (α-Al_2O_3) in the form of flat, optically smooth 4 inch discs, was obtained from Silica Source Technology, Temple, Arizona, USA.

Ballotini (glass) spheres (60-200μm in diameter) were obtained from Ceramic Industries (Victoria, Australia). The sample was fractionated to allow us to separate out only the largest of the particles for column separation investigations. The rational is that by producing a packed column of silica of sufficiently large size, the pores between the individual spheres would be large compared with the size of *Cryptosporidium* oocyst (see Figure 4) and therefore any *Cryptosporidium* retention would be solely due to an adsorption process rather than a

physical retention based on a size exclusion alone. The Ballotini spheres were dispersed in aqueous solution, shaken and quickly allowed to settle, the fine dispersed phase was decanted off and the remaining material redispersed over and over, repeatedly (20x) discarding the dispersed material until only the largest of particles were retained.

Alumina powder (63-200μm diameter) was obtained from Merck (art. 1077). The alumina sample was fractionated to allow us to separate out only the largest of the particles for column separation investigations. The rational is that by producing a packed column of alumina of sufficiently large size, the pores between the individual spheres would be large compared with the size of *Cryptosporidium* oocyst (see Figure 4) and therefore any *Cryptosporidium* retention would be solely due to a chemisorption process rather than a physical retention based on a size exclusion alone. The alumina was dispersed in aqueous solution, shaken and quickly allowed to settle, the fine dispersed phase was decanted off and the remaining material redispersed over and over, repeatedly (20x) discarding the dispersed material until only the largest particles were retained.

Adsorption Studies

(1) Alumina substrates, immediately after cleaning using UV irradiation, were directly exposed to 100μl of a 5 x 10^6 oocysts/ml *Cryptosporidium* solution. The treated substrates were placed into a covered petrie dish housed inside another larger moist petrie dish in a refrigerator for several hours allowing oocysts to adsorb to the positively charged surface. After this time the liquid was decanted off and the substrate was rinsed with distilled water. Any residual liquid removed by way of capillary action using filter paper. The substrate were then examined under a Kombistereo Wild M32 light microscope having a magnification range 162-1000X using a Intralux 5000 optical fibre light source. The adsorption density was photographed with a Kodak 400 flexi clear film at a film speed of 200 ASA using a Nikon camera.

(2) Fluorspar (fluorite, CaF_2) plates were cleaned by consecutive washings of ethanol and distilled water to remove any surface active contaminants. The plates were then exposed to 50μl of a 5 x 10^6 oocysts/ml *Cryptosporidium* solution. The treated substrates were placed into a covered petrie dish housed inside another larger moist petrie dish in a refrigerator for several hours allowing oocysts to adsorb to the positively charged surface. After this time the liquid was decanted off and the substrate was rinsed with distilled water. Any residual liquid was removed by way of capillary action using a filter paper. The substrates were then examined under a Kombistereo Wild M32 light microscope having a magnification range 162-1000X using a Intralux 5000 optical fibre light source. The adsorption density was photographed with a Kodak 400 flexi clear film at a film speed of 200 ASA using a Nikon camera.

Column Separations

(1) Ballotini spheres (silica spheres) (60-200μm diameter) was obtained from Ceramic Industries (Victoria, Australia). The spheres were dispersed into aqueous solution, shaken and quickly allowed to settle, the fine dispersed phase was decanted off and the remaining material redispersed over and over, repeatedly (20x) discarding the dispersed material until only the largest of particles were retained. A slurry of this retained Ballotini spheres was transferred to a glass chromatography column (28mm O.D) containing a #3 glass sinter the excess liquid was run from the column leaving a packed column having a depth of 5mm (see Figure 5). A 100μl of the *Cryptosporidium* sample at 3 x 10^8 oocysts/ml was diluted to 25mls in a volumetric flask, 10ml of this solution was then transferred to the packed silica column, and the solution allowed to stand 15 minutes before being allowed to percolate through the silica at a rate (10ml/hr) into glass vials. After the entire contents had come through, the column was washed three times with 10ml of distilled water allowing it to percolate through at a similar rate to that of the original *Cryptosporidium* sample. Each 10ml fraction was collected, transferred to a microelectrophoresis cell and examined using dark field illumination. This allowed us to ascertain the density and charge of any particles

present.

(2) Alumina powder (63-200μm diameter) was obtained from Merck (art. 1077). The powder was dispersed into aqueous solution, shaken and quickly allowed to settle, the fine dispersed phase was decanted off and the remaining material redispersed over and over, repeatedly discarding the dispersed material until only the largest of particles were retained. A slurry of this retained alumina powder was transferred to a glass chromatography column (28mm O.D) containing a #3 glass sinter the excess liquid was run from the column leaving a packed column having a depth of 5mm (see Figure 5). A 100μl of the *Cryptosporidium* sample at 3 x 10^8 oocysts/ml was diluted to 25mls in a volumetric flask, 10ml of this solution was then transferred to the alumina packed column, and the solution allowed to stand 15 minutes before being allowed to percolate through the alumina at a rate (10ml/hr) into glass vials. After the entire contents had come through, the column was washed twice times with 10ml of distilled water allowing it to percolate through at a similar rate to that of the original *Cryptosporidium* sample. Each 10ml fraction was collected, transferred to a microelectrophoresis cell and examined using dark field illumination. This allowed us to ascertain the density and charge of any particles present.

Example 1

Direct Adsorption on Alumina

The *Cryptosporidium* oocyst sample received from AWT was 5 x 10^6 oocysts/ml. Since the oocysts are approximately 5μm in diameter, the area per oocyst of 1.9 x 10^{-7}cm^2 means the number of oocysts required to cover a 1cm^2 substrate is 5 x 10^6. In these studies we have used 0.1ml samples of the original 5 x 10^6 oocysts/ml sample.

Pure alumina (α-Al_2O_3) wafers were cut to an appropriate size and UV irradiated at (λ185 and 254mm) for 1hr in the presence of water vapour to remove any organic contamination.

The UV irradiation in the presence of water vapour and oxygen produces ozone and hydroxyl radicals which clean the alumina surfaces rendering them hydrophilic. After UV irradiation a small droplet of distilled water was placed on the substrates to ensure the surfaces were hydrophilic, then blown dry under a gentle stream of nitrogen.

The blank substrates were examined under a Kombistereo Wild M32 light microscope having a magnification range 162-1000X. The alumina wafer was highly polished and so only dust contamination and the roughness of the reverse side of the wafer enabled us to focus on the smooth surface. Figure 6 is a photograph of the microscope focussed on the optically smooth surface having a few extraneous dust particles attached. This indicated that the wafer would provide an excellent, optically smooth surface for adsorption investigations using light microscopy.

The UV treated (hydrophilic) alumina wafer was directly exposed to 0.1ml of a 5×10^6 oocysts/ml solution. The treated substrate was placed into a covered petrie dish housed inside another larger moist petrie dish in a refrigerator for several hours allowing oocysts to adsorb to the positively charged surface. After this time the liquid was decanted off the substrate and any residual liquid removed by way of capillary action using a filter paper. The substrate was examined in a similar manner to that of the blank. When focussed on the alumina surface, a uniform layer of about 1/10th monolayer coverage of oocysts was observed (see Figure 3) which is consistent with the original oocyst density (i.e. 5×10^5 oocysts), suggesting that all or most of the oocysts were recovered from solution by direct adsorption to the alumina substrate.

To test whether adsorbed oocysts could be easily desorbed, we placed the coated alumina substrate into a covered beaker containing distilled water for approximately 20 hours. After this time, the substrate was taken out with the aid of tweezers and any residual liquid removed via capillary action using a filter paper, it was then examined under the microscope. The

adsorption density of oocysts was unchanged, indicating that the adsorbed oocysts were stable in water at pH 5.7. This result is consistent with the observation that alumina is positively charged at this pH. Furthermore, it also indicates that *Cryptosporidium* oocysts have a high affinity for the alumina surface.

To test whether the oocysts would remain adsorbed to alumina substrates at higher pH values, we placed the coated alumina into buffer pH 9.2. At this pH the alumina becomes negatively charged which might cause oocyst desorption if the adsorption was purely electrostatic in origin.

However, after leaving the coated substrate for 16 hours at this pH the substrate was examined under the microscope, again no oocyst desorption was found to occur. This could be either because at this pH the (negative) surface potential is still too weak to overcome the strong van der Waals attraction or because once the oocysts have adsorbed, strong short range ligand bonds are formed between the surface aluminium and the carboxylate or phosphate groups present on the oocyst surface. To test whether the low surface potential could be the reason for the failure to desorb, we placed the coated substrate in a pH 10 buffer (where the alumina should acquire a high negative surface potential) for several hours, again no oocyst desorption was observed. This provides further evidence that the resultant oocyst adsorption was due to a relatively strong chemisorption.

Example 2

Direct Adsorption on Fluorspar

The *Cryptosporidium* oocyst sample received from AWT was 5 x 10^6 oocysts/ml. A 50μl sample of the original 5 x 10^6 *Cryptosporidium* oocysts/ml sample were used for the initial fluorspar adsorption studies.

Fluorspar is an ideal substrate as it is positively charged up to around pH 7.8 [2] and the extent of adsorption could be easily estimated on polished samples via optical microscopy.

Fluorspar (fluorite, CaF_2) was cleaned by consecutive washings of ethanol and distilled water as to remove any surface active contaminant. It was then exposed with 50μl of a 5 x 10^6 oocysts/ml *Cryptosporidium* solution. The treated substrates were placed into a covered petrie dish housed inside another larger moist petrie dish in a refrigerator for several hours allowing oocysts to adsorb to the positively charged surface. After this time the liquid was decanted off the substrate, which was then rinsed with distilled water and any residual liquid removed by way of capillary action using a filter paper. The substrate were then examined under a Kombistereo Wild M32 light microscope having a magnification range 162-1000X using an Intralux 5000 optical fibre light source. A uniform *Cryptosporidium* oocyst adsorption was evident and the adsorption density photographed with a Kodak 400 flexi clear film at a film speed of 200 ASA using a Nikon camera.

The substrate was placed back into distilled water to determine the stability of the oocyst adsorption. The plate was continuously examined over the next week, no desorption was found to occur. Knowing the isoelectric point for fluorspar was 7.8, we tested whether the adsorption was purely electrostatic in origin by placing the substrate overnight in buffer 9.2, again no desorption was found to occur. It was also placed into pH 10 buffer and again no desorption was found to occur, suggesting the adsorption was not electrostatic in origin. This suggested the resultant adsorption was due to a chemisorption process taking place at the substrate surface.

The reversibility of this adsorption was investigated by placing the adsorbed plate into a 0.1M EDTA (Ethylene Diamine Tetra Acetic acid) solution overnight, because EDTA competes for the calcium complexed to the oocysts carboxylates or phosphates it would allow us to determine whether the complex formed with the oocysts is more favourable than the

competing calcium-EDTA complex. No desorption was found to occur indicating the oocyst calcium complex was stable and irreversible.

It was previously reported [3] that fluorspar can be made "hydrophilic" (water loving) or "hydrophobic" (water hating) depending on bathing electrolyte. That is fluorspar is known to be "hydrophilic" in the presence of 0.1M NaF and "hydrophobic" in the presence of 0.1M NaCl. To test whether the adsorption was due to a hydrophobic interaction existing between the oocyst and the fluorspar substrate, the adsorbed plate was placed into 0.1M NaF solution. In principle, if the adsorption had been a result of hydrophobic attraction then when the substrate was rendered hydrophilic the adsorbed oocysts should desorb. This was found not to be the case, no desorption was found to occur.

It appears fluorspar is a good substrate for *Cryptosporidium* oocyst adsorption. The adsorbed oocyst layer appears to be robust, stable and totally resistant to desorption with pH, EDTA and NaF treatment. *Cryptosporidium* adsorption appears to be due to both an electrostatic attraction and chemisorption between the surface calcium ions and either the carboxylate or phosphate groups present on the oocyst surface.

Example 3

Column Separators

A glass chromatography column (28mm O.D) containing a #3 glass sinter was chosen for column separations (see Figure 5). In order to ensure the pores of the glass sinter were large enough to allow the *Cryptosporidium* oocysts through, we took a 10ml sample of 1.5×10^6 oocysts/ml allowed it pass through the glass sinter of the unpacked column.

The column permeate was collected and transferred to a microelectrophoresis cell and examined using dark field illumination. At the first stationary level about 125 negatively

charged particles (oocysts) were observed. This demonstrated that oocysts could easily pass through the glass sinter.

(2.2) Silica Blank

The silica (Ballotini) spheres were fractionated to a size approx 200μm in diameter as described earlier. The glass column was packed to a depth of 5mm with the fractionated large Ballotini spheres. 10ml of distilled water was allowed to percolate through the packed column and the permeate collected. The permeate was transferred to a microelectrophoresis cell. This not only allowed us to test whether the sinter was of sufficient porosity to retain the silica support but it also allowed us to determine the background levels. The sinter was found to adequately retain the packing support, since the permeate typically had about 8 negatively charged particles in the field of view.

(2.3) Silica Packed Column

Ballotini spheres (60-200μm diameter) were obtained from Ceramic Industries (Vic. Aust.). The spheres were dispersed in to aqueous solution, shaken and quickly allowed to settle, the fine dispersed phase was decanted off and the remaining material redispersed over and over, repeatedly discarding the dispersed material until only the largest of particles were retained (approx. 20X). A slurry of this retained Ballotini spheres was transferred to a glass chromatography column (28mm O.D) containing a #3 glass sinter the excess liquid was run from the column leaving a packed column having a depth of 5mm. A 100μl of the *Cryptosporidium* at 3 x 10^8 oocysts/ml was diluted to 25mls in a volumetric flask, 10ml of this solution was then transferred to the packed silica column, and the solution allowed to stand 15 mins before being allowed to percolate through the silica at a rate (10ml/hr) into glass vials. After the entire contents had come through, the column was washed three times with 10ml of distilled water allowing it to percolate through at a similar rate to that of the original *Cryptosporidium* sample. Each 10ml fraction was collected, transferred to a microelectrophoresis cell and examined using dark field illumination.

The *Cryptosporidium* sample before passing through the packed support contained about 75-78 negatively charged particles at a given plane of view within the cell. The permeate which was passed through the packed column was seen to have about 70 negatively charged particles. Three further 10ml washings were put through the column to see whether the *Cryptosporidium* would desorb or whether there was a lag time involved in total *"crypto"* recovery. The first, second and third washings were found to contain 34, 13 and 8 negatively charged particles, respectively. The results obtained are shown graphically in Figure 7. This demonstrates that *Cryptosporidium* was not retained in the column support (i.e. no adsorption evident) thus allowing the oocysts to travel through large intra-pore spacing between adjacent silica particles.

Recent work by Jerry Ongerth [4] suggested *Cryptosporidium* could be removed via a diatomaceous earth (silica) filter. Our results suggest that the *Cryptosporidium* removal reported in this paper was based purely on the principle of size exclusion due to the intrapore spacing of the diatomeous earth used. Silica has a net negative charge similar to that of *Cryptosporidium*, therefore based purely on electrostatics there is no reason to expect any *Cryptosporidium* adsorption. From our results presented here we have strong evidence to suggest silica (diatomaceous earth) can only work as a filter based on a physical size exclusion.

(2.4) Alumina Blank

The alumina powder was fractionated to a size approximately 200μm in diameter as described in materials and methods. The glass column was packed to a depth of 5mm with the fractionated alumina powder. 10ml of distilled water was allowed to percolate through the packed column and the permeate collected. The permeate was transferred to microelectrophoresis cell and set at the first stationary level. This not only allowed us to test whether the sinter was of sufficient porosity to retain the alumina support but it also allowed us to determine background levels. The sinter was found to adequately retain the packing

support, since the permeate typically had about 14 negatively charged particles at any plane of view.

(2.5) Alumina Packed Column

Alumina powder (63-200μm diameter) was obtained from Merck (art. 1077). The powder was dispersed in to aqueous solution, shaken and quickly allowed to settle, the fine dispersed phase was decanted off and the remaining material redispersed over and over, repeatedly discarding the dispersed material until only the largest of particles were retained. A slurry of this retained alumina powder was transferred to a glass chromatography column (28mm O.D) containing a #3 glass sinter. The excess liquid was run from the column leaving a packed column having a depth of 5mm. A 100μl of the *Cryptosporidium* at 3 x 10^8 oocysts/ml was diluted to 25mls in a volumetric flask, 10ml of this solution was then transferred to the alumina packed column, and the solution allowed to stand 15 mins before being allowed to percolate through the alumina at a rate (10ml/hr) into glass vials. After the entire contents had come through, the column was washed twice with 10ml of distilled water allowing it to percolate through at a similar rate to that of the original *Cryptosporidium* sample. Each 10ml fraction was collected, transferred to a microelectrophoresis cell and examined using dark field illumination. This allowed us to ascertain the density and charge of any particles present.

The *Cryptosporidium* sample before passing through the packed support contained about 70 negatively charged particles at a given plane within the cell. By comparison, the permeate which was passed through the packed column was seen to have only 4 negatively charged particles (i.e below background level) at the given plane. Two further 10ml washings were put through the column to see whether the *Cryptosporidium* could be easily desorbed. The second and third washings were found to contain only 1 negatively charged particle (see Figure 8).

This demonstrated that *Cryptosporidium* was retained in the column due to adsorption, most likely via a combination of electrostatic forces and chemisorption as the intra-pore spacing between adjacent alumina particles would have presented no barrier based on size exclusion alone. Also, repeated washings provided good evidence that the oocysts could not be easily desorbed.

Future processes using fluorspar in *Cryptosporidium* removal could either incorporate the use of a packed cartridge of powdered material or use a direct precipitation/coagulation process producing insoluble CaF_2 flocs in-situ.

From column separation experiments using silica and alumina supports we have found that silica shows no selective adsorption for *Cryptosporidium* oocysts. Recent reports suggesting that diatomaceous earth (porous silica) can remove *Cryptosporidium* are most likely based entirely on its filtration properties due to size exclusion. By comparison, *Cryptosporidium* oocysts appear to have a high affinity for the alumina surface.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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Figure 1

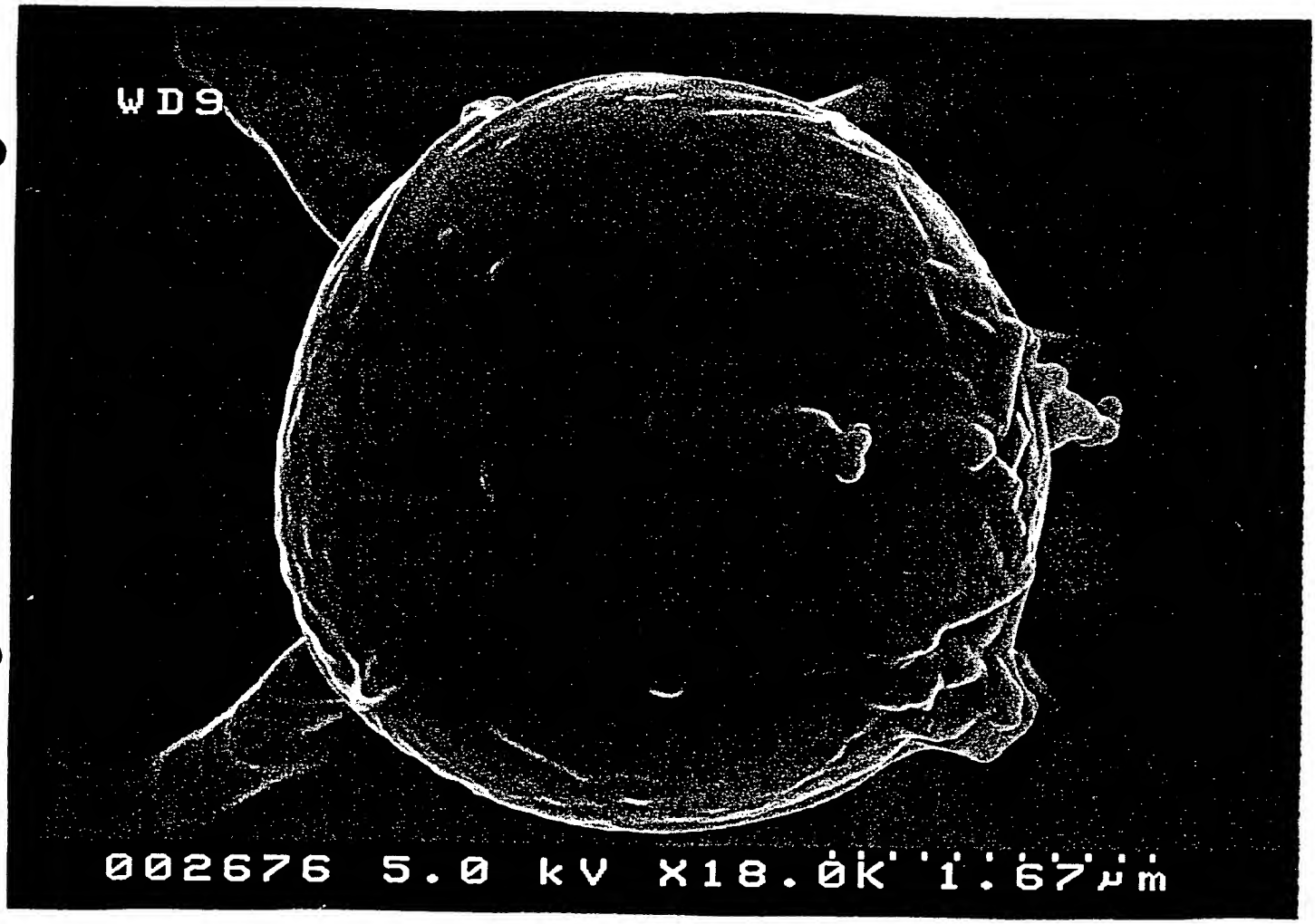

Figure 2

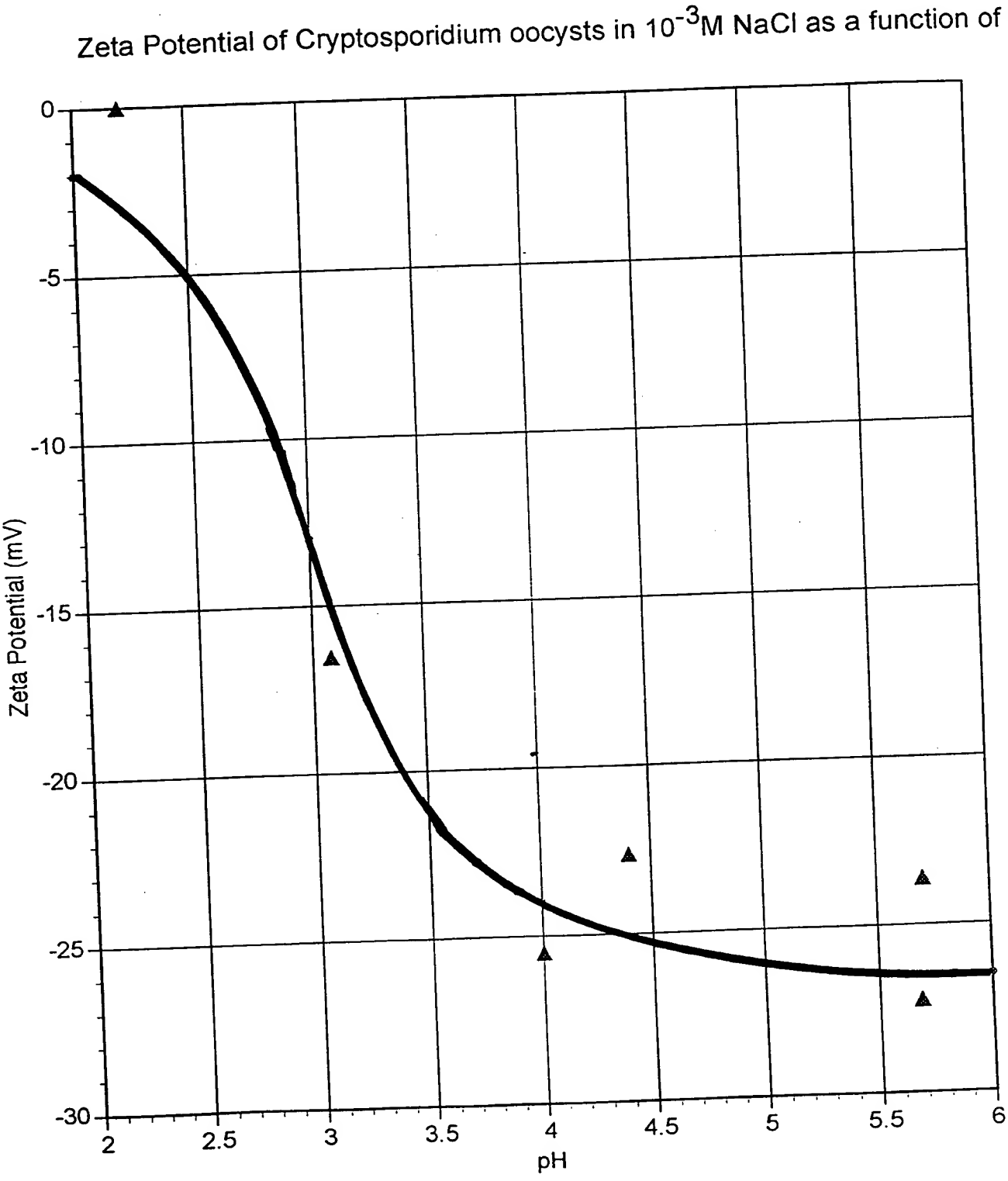
Zeta Potential of Cryptosporidium oocysts in 10^{-3}M NaCl as a function of pH
Zeta Potential (mV)
0
-5
-10
-15
-20
-25
-30
2
2.5
3
3.5
4
4.5
5
5.5
6
pH

Figure 3

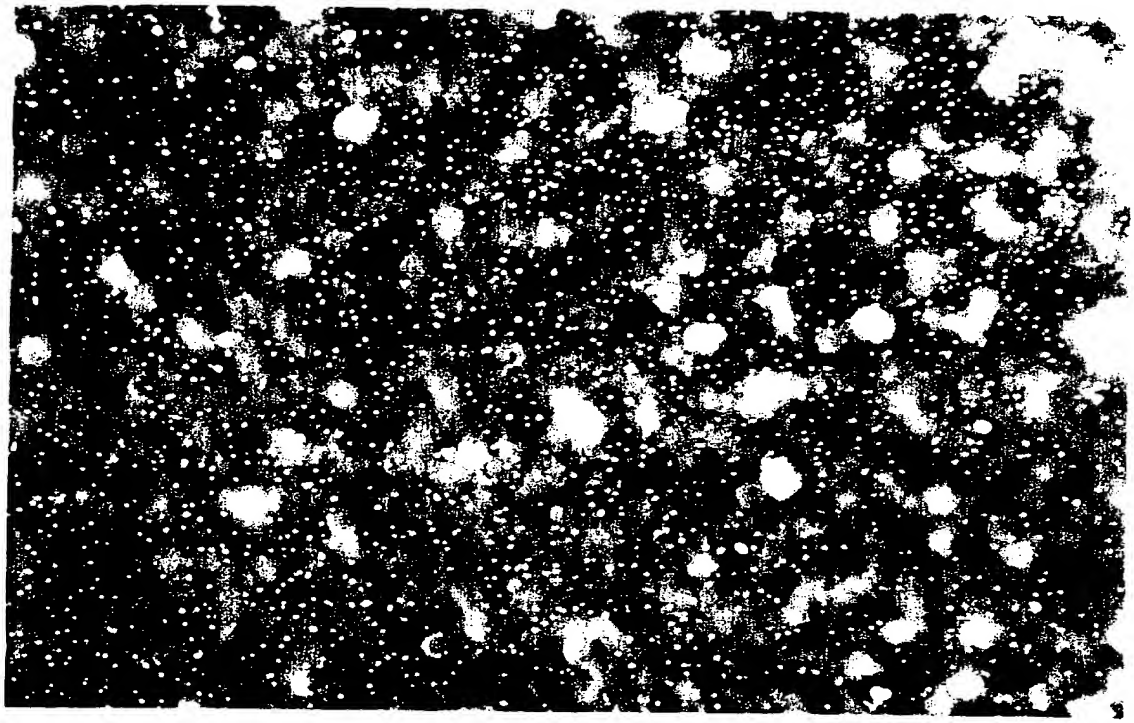

Figure 4

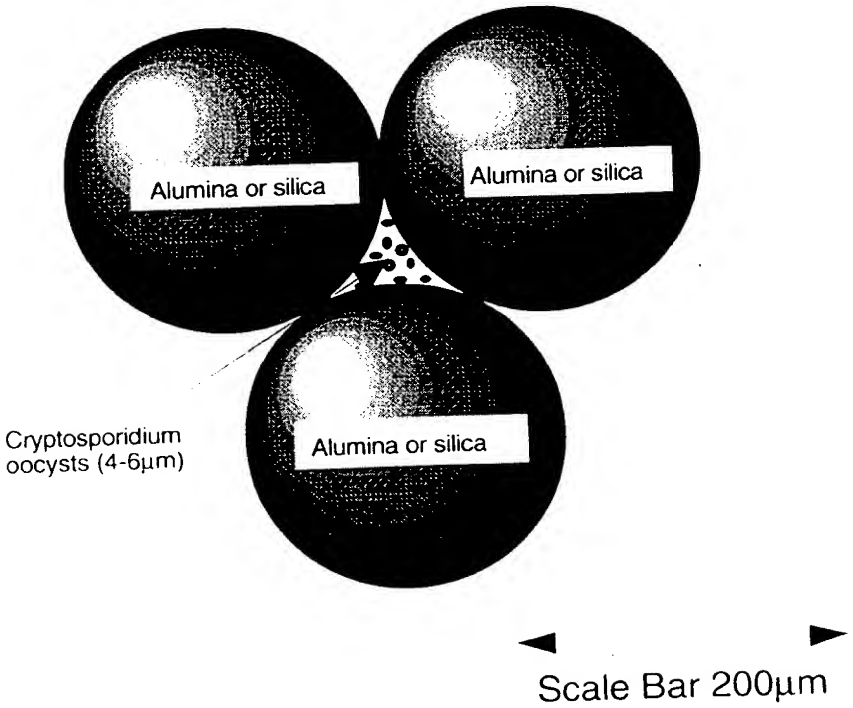
Alumina or silica
Alumina or silica
Alumina or silica
Cryptosporidium
oocysts (4-6µm)
Scale Bar 200µm

Figure 5

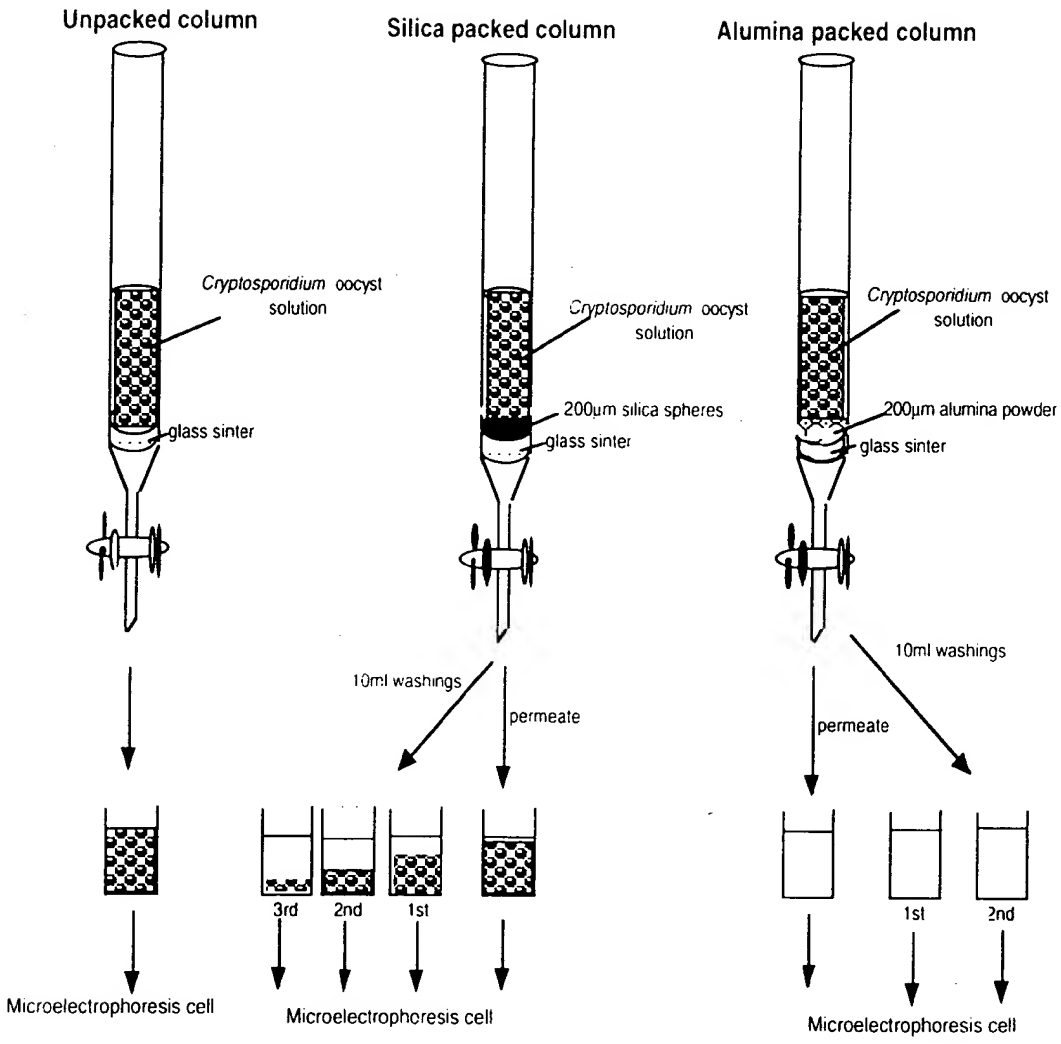
Unpacked column
Cryptosporidium oocyst solution
glass sinter
Microelectrophoresis cell
Silica packed column
Cryptosporidium oocyst solution
200µm silica spheres
glass sinter
10ml washings
permeate
3rd
2nd
1st
Microelectrophoresis cell
Alumina packed column
Cryptosporidium oocyst solution
200µm alumina powder
glass sinter
10ml washings
permeate
1st
2nd
Microelectrophoresis cell

Figure 6

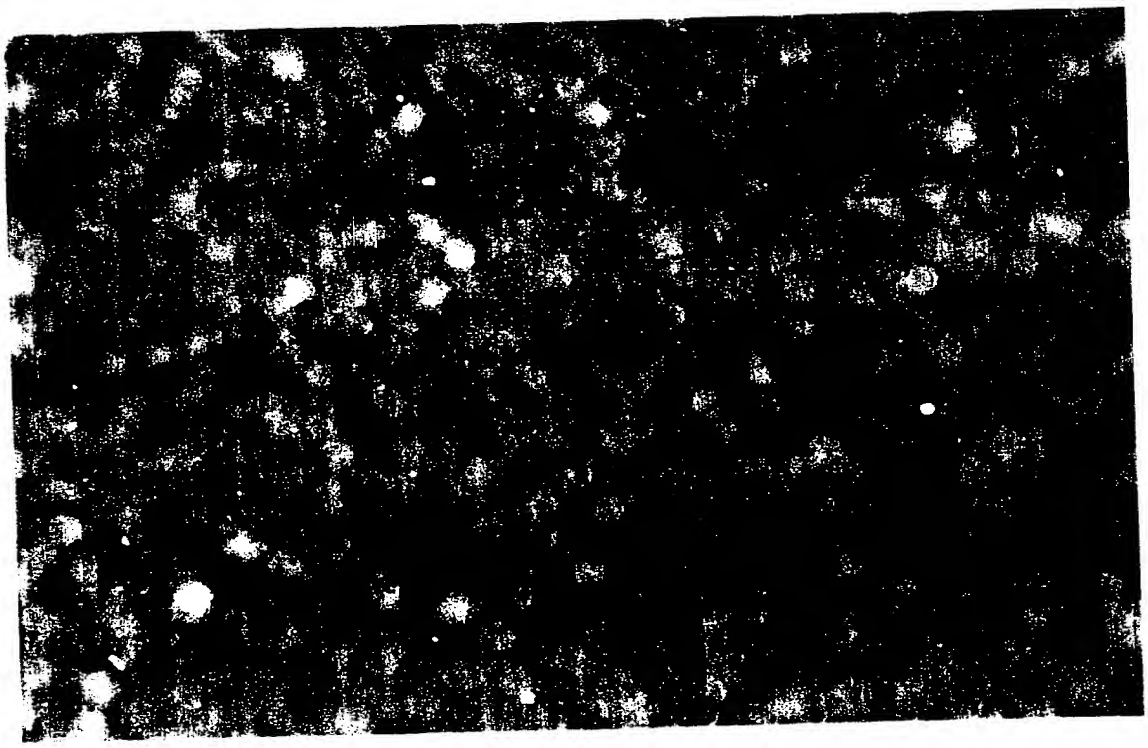

Figure 7

Silica column investigation

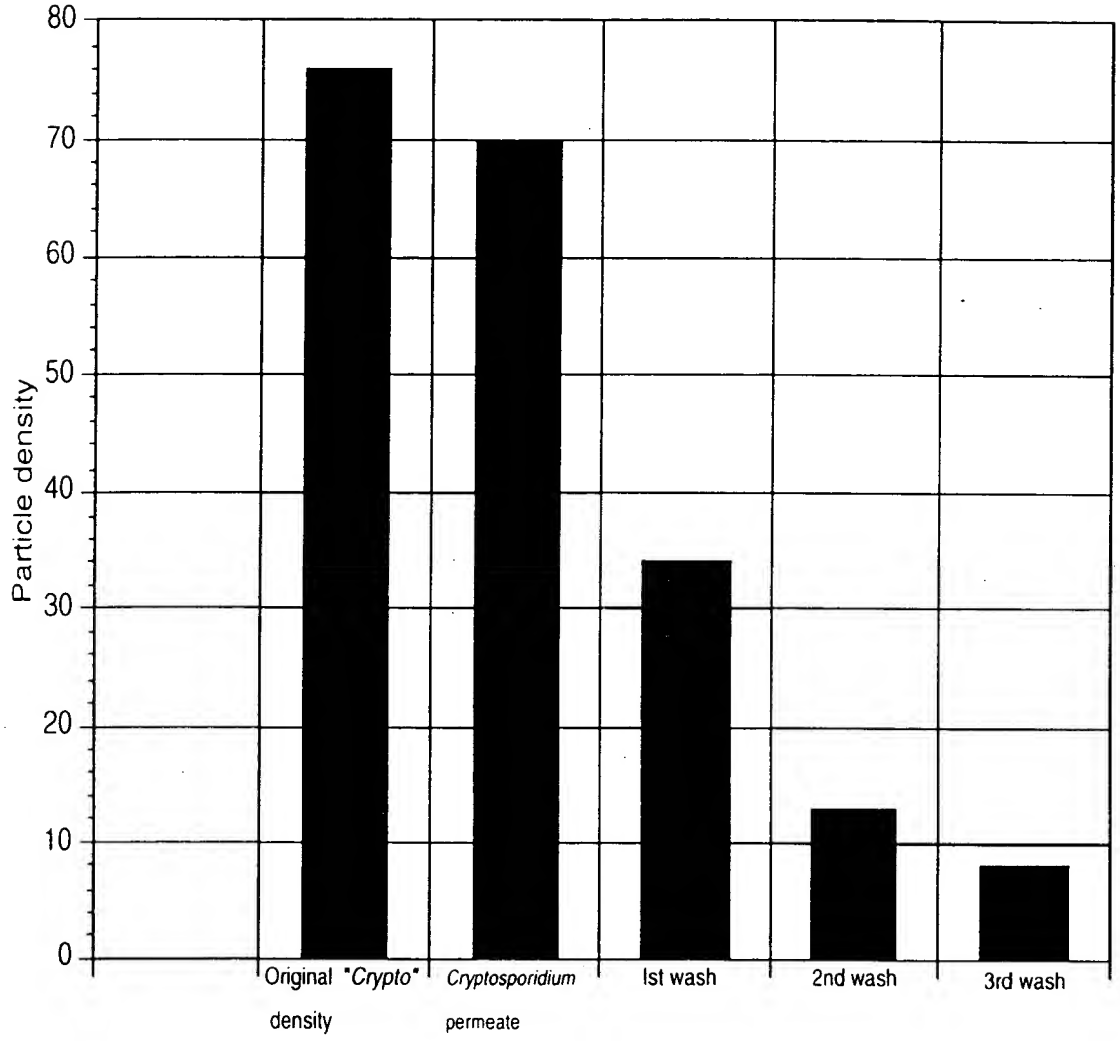

Figure 8

Alumina column investigations